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Regulation of hormone-sensitive lipase expression by saturated fatty acids and hormones in bovine mammary epithelial cells ☆

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ABSTRACT

Hormone-sensitive lipase was firstly identified as an epinephrine-induced lipase in adipocyte. HSL mRNA was detected by RT-PCR in cloned bovine mammary epithelial cells (bMEC) and bovine lactating mammary gland. Saturated fatty acids (stearate and palmitate), but not unsaturated fatty acids (oleate and linoleate) induced up-regulation of HSL mRNA in a time- and concentration-dependent manner in bMEC. Treatment with insulin (5–10 ng/ml), dexamethasone (50–250 nM) or GH (50 ng/ml) induced down-regulation of HSL. These results suggest that HSL was regulated by fatty acids and some hormones in mammary epithelial cells and thereby play an important role of lipid and energy metabolism.

During lactation, the energy metabolism of mammary epithelial cells is dramatically changed in order to enable the synthesis of the milk components [1]. Lactating animals dramatically diminish the storage of nutrients, particularly of triacylglycerol (TAG), in adipose tissue. The lactating mammary gland thus utilizes fatty acids which are derived from dietary fats and TAG released from the adipose tissue. Lactating mammary epithelial cells (MEC) are also highly active in the synthesis of fatty acids de novo. Each gram of mammary gland tissue is known to produce 1–2 ml of milk per day, and 2–600 g of fat/l milk [2]. Thus, mammary epithelial cells have the ability to produce a remarkable amount of lipid. Nevertheless, little is known about lipid metabolism in these cells.

HSL was firstly identified as an enzyme which has an epinephrine-induced lipolytic activity in the adipose tissue and was also activated by lipolytic hormones such as catecholamine, isopreterenol, glucagons and ACTH [3]. The activation is regulated by a cAMP-related pathway, whilst the suppression is caused by insulin [3]. In addition, HSL has catalytic activity for triacylglycerols, diacylglycerols, monoacylglycerols, cholesterol esters, and retinol esters, but not phospholipase [3]. A recent study has demonstrated that HSL protein and activity exist in milk [4] and that HSL protein and activity are negatively correlated with plasma insulin concentrations [5].

In this study, we have demonstrated that the cloned bovine mammary epithelial cell (bMEC) and lactating mammary gland express HSL mRNA, and that saturated fatty acids activate the expression of HSL mRNA in these cells. We also investigated the effects of some hormone on the expression of HSL mRNA.

Materials and methods

Materials. Long-chain fatty acid sodium salts (palmitate, stearate, oleate and linoleate), insulin, dexamethasone, Dulbecco's modified Eagle's medium (DMEM), fetal calf serum (FCS) and fraction V fatty acid-free bovine serum albumin (BSA) were purchased from Sigma–Aldrich, St. Louis, MO. TRIsol and SuperScript III First-Strand Synthesis System were from Invitrogen Corp., Carlsbad, CA. DyNAmo SYBR green qPCR Kit were from Finnzymes, Espoo, Finland. Deoxyribonuclease (RT Grade) for Heat Stop was from Nippon Gene Co., LTD., Tokyo, Japan. Bovine growth hormone (GH) was from Monsanto, St. Louis, MO, USA.

Cell cultures. Bovine mammary epithelial cell (bMEC) were isolated from the mammary gland of a 102-day pregnant Horstein heifer and cloned using the limiting dilution method [6]. FACS analysis revealed that all the cells was positively stained using anti-cytokeratin antibody. Additionally the cells possess the potential to produce a milk protein, an α-casein, which is dependent on the presence of lactogenic hormone cocktails [6]. The cells were seeded at 1×10^4 cells/cm^2 in a collagen coated 6-well plate and 100-mm dish (Corning, NY) and grown in DMEM supplemented with 10% FCS, 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin under 5% CO_2 and air at 37 °C until confluency.

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RNA preparation and RT-PCR analysis. The principal methods for total RNA extraction and RT-PCR were described in our previous paper [7]. RT-PCR primers for bovine HSL were designed from bovine HSL mRNA (Accession No. U78042). The sequence of the primers are as follows: bovine HSL forward, 5′-GCCTGAAGCTGAA TGACCTG-3′ bovine HSL reverse, 5′-CAGCGGTGACATGAAGGGAT-3′ The PCR reaction was performed using KOD plus DNA polymerase and template with 1 μl of the RT product. Forty PCR cycles were carried out at 94 °C for 15 s (denaturing), 52 °C for 30 s (annealing), and 68 °C for 1 min (extension). The expected amplicon size for bovine HSL was 299 bp. The product was subcloned into sequence vector and sequenced to confirm the identity of bovine HSL. RT-PCR primers for bovine GAPDH was used as described previously [7].

Quantitative RT-PCR analysis. RT products were prepared as we previously described [7]. Primers were designed from bovine HSL and GAPDH mRNA (Accession Nos. AJ237675 and U85042, respectively). Quantitative-RT-PCR was performed using following primer sets: bovine HSL forward, 5′-CGGGAGGCTCTTCTTTGAGG-3′ bovine HSL reverse, 5′-TGGAGATGGTCTGCAGGAAC-3′ bovine GAPDH forward, 5′-TCAACGGGAAGCTCACTGG-3′ bovine GAPDH reverse, 5′-CCCCAGCATCGAAGGTAGA-3′ using an SYBR Green qPCR kit and DNA Engine Opticon 2 Continuous Fluorescence Detector (MJ Research Inc., MA, USA). The expected amplicon sizes for bovine HSL and GAPDH were 146 bp and 225 bp, respectively. The reactions were performed as described previously [7]. The PCR reaction was run for 40 cycles using the following protocol: 10 s denaturation at 95 °C; 20 s annealing at 60 °C; 20 s extension at 72 °C. Post-PCR melting curves confirmed the specificity of the single-target amplification and the expression of each gene relative to GAPDH was determined in triplicate.

Statistical analysis. Values are expressed as the means ± SEM of at least triplicate samples in each experimental group; experiments were replicated to ensure consistency. Statistical significance was determined using a one-way ANOVA followed by Bonferroni's multiple range test. Values were considered to be statistically significant if their P value was <0.05.

Results

Expression of bovine hormone-sensitive lipase in bovine mammary epithelial cells and lactating mammary gland

Bovine HSL mRNA expression in bMEC and lactating bovine mammary gland (Peak lactation) were evaluated using RT-PCR analysis with sets of specific primers and were detected as the expected size in bMEC and lactating mammary gland (Fig. 1A left lane). No PCR product was detected in the absence of reverse transcriptase (Fig. 1A right lane). The PCR product was cloned and sequence analysis showed 100% homology with bovine HSL mRNA. Bovine GAPDH primer designed from the region containing an intron did not amplify any products except a single 299 bp product, indicating no contamination of genomic DNA (Fig. 1A, lane1).

Saturated LCFA stimulates the expression of HSL mRNA

We determined the expression of HSL mRNA using quantitative-RT-PCR in bMEC incubated with LCFAs. Incubation with 400 μM palmitate and stearate, but not oleate and linoleate, for 24 h markedly increased HSL mRNA expression (Fig. 1 B). The values were 7.8- and 5.0-fold of control levels for palmitate, and stearate, respectively (Fig. 1B). To examine whether the effect of saturated LCFAs on HSL mRNA expression has a biological significant in bMEC, we measured the levels of HSL mRNA expression following the treatment of these cells with various concentration of

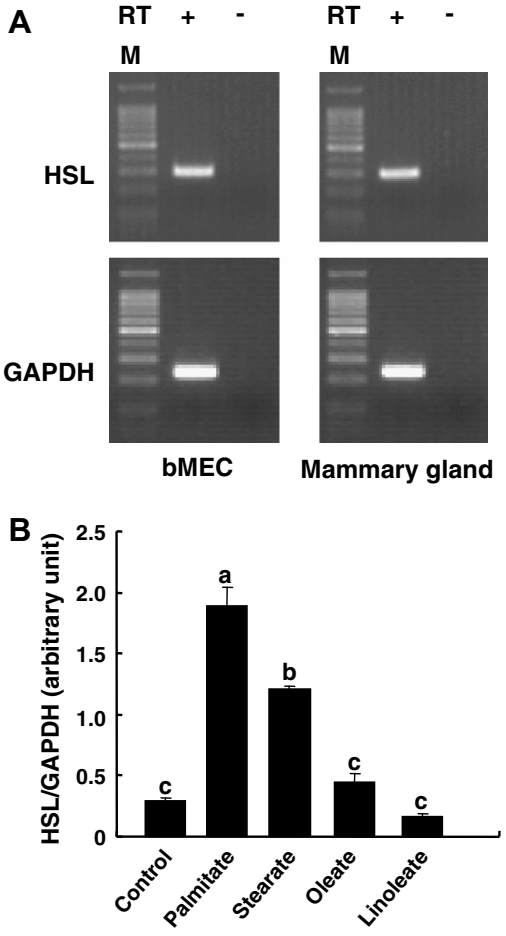

Fig. 1. Bovine HSL mRNA expression in bMEC and bovine lactating mammary gland (Peak lactation). RT-PCR was performed as described in the Materials and methods. The products were run on a 2% agarose gel stained with ethidium bromide. (A) To exclude amplification from genomic DNA contamination, the RNA was either treated with DNase before the RT reaction (RT+) or run without RT (negative control, RT−). A 100 bp DNA step ladder appears in the left lane as a molecular size marker. Effects of LCFAs on the expression of bovine HSL mRNA in bMEC. The cells were treated with 400 μM each LCFAs bound to 0.5% BSA for 24 h. (B) The cDNA produced by reverse transcription from 5 μg of total RNA that were extracted from the cells were analyzed by quantitative-RT-PCR. The relative values of bovine HSL mRNA to GAPDH mRNA are shown. The data are expressed as the means ± SEM (n = 3–4). The values without associated common letters are significantly different (P < 0.05, Bonferroni's multiple range test).

LCFAs. Palmitate and stearate, but not oleate and linoleate, stimulated the expression of HSL mRNA in a concentration dependent manner (Fig. 2A). We also examined the change of HSL mRNA expression for 24 h incubation with LCFAs. At all occasions, stearate induced a significant increase in the expression of HSL mRNA compared to control, and palmitate also significantly increased the expression except at 6 h (Fig. 2B). Unsaturated (oleate and linoleate), however, caused a significant suppression in the expression of HSL mRNA at 12 and 18 h (Fig. 2B)

Insulin, dexamethasone and growth hormone suppress the expression of HSL mRNA

We used quantitative-RT-PCR to examine the effects of the hormones insulin, dexamethasone, and bovine growth hormone on bMEC. Incubation with insulin (5–10 ng/ml) or dexamethasone (50–250) suppress the expression of HSL mRNA in a concentration dependent manner (Fig. 3A and B). The percentages of suppression in expression in cells incubated with insulin 5

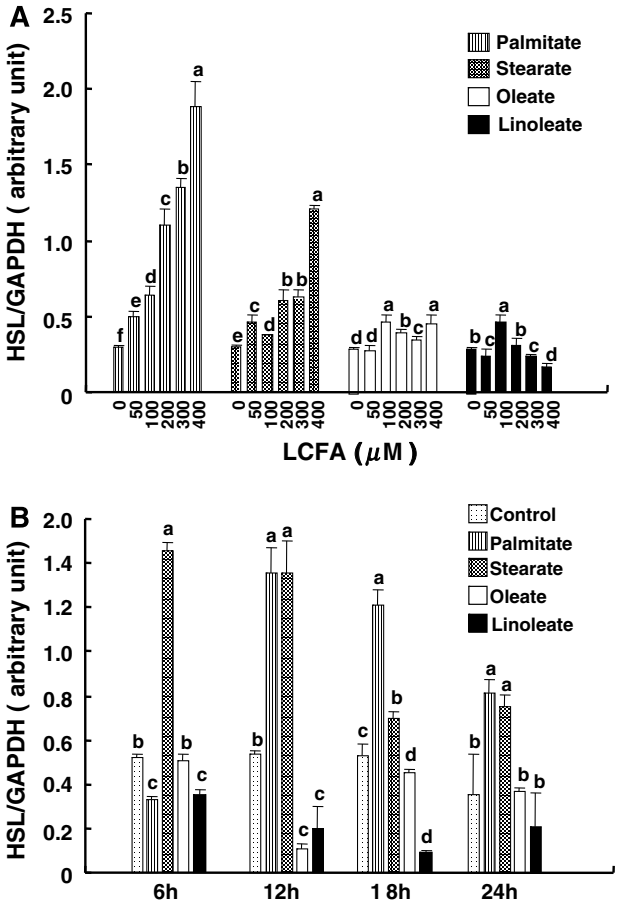

Fig. 2. Changes of the levels of HSL mRNA by LCFAs in a concentration- and time-dependent manner. (A) The cells were treated with various concentrations of LCFAs for 24 h. (B) The cells were treated with 400 μM each LCFAs bound to 0.5% BSA at various incubation period. The cDNA produced by reverse transcription from 5 μg of total RNA that were extracted from the cells were analyzed by quantitative-RT-PCR. The relative values of bovine HSL mRNA to GAPDH mRNA are shown. The data are expressed as the means ± SEM ($n = 3–4$). The values without associated common letters are significantly different ($P < 0.05$, Bonferroni's multiple range test).

and 10 ng/ml relative to control were 61% and 97%, respectively. These in cells incubated with dexmethasone 50, 100 and 250 μM relative to control were 57%, 72% and 97%, respectively. Incubation with 100 ng/ml bGH significantly suppress the expression of HSL mRNA (Fig. 3C).

Discussion

The present study, for the first time, demonstrated that HSL mRNA expression in mammary epithelial cells is elevated following stimulation with saturated LCFAs. Saturated fatty acids, but not unsaturated, markedly induced the elevation of HSL mRNA expression in a concentration-dependent manner. A study with an adipocyte cell line showed that reducing the concentration of glucose in the media bathing cells decreased the expression of HSL mRNA, which was reversed by the re-introduction of glucose, but not with oleate, linoleate or linolenate [8]. Additionally, palmitoyl-CoA stimulates the activity of HSL in islets β-cell [9], while palmitoyl- and oleoyl-CoA suppress in adipocyte [9,10]. This indicates that response of mammary epithelial cell to saturated LCFAs is different from that of other cells.

In rat primary cultured adipocytes, insulin increased HSL activities and proteins in a glucose concentration-dependent manner [11,12]. In lactating rat mammary gland, the levels of HSL protein and activity are negatively correlated with plasma insulin concentrations [5]. We further demonstrated that physiological concentration of insulin suppresses the expression of HSL using in vitro culture model. In human adipose tissue explants, GH and dexamethasone enhance the activity and the expression of HSL [13]. In rat primary culture adipocyte, dexamethasone stimulates the expression of HSL mRNA in a concentration-dependent manner [14]. We have demonstrated for the first time that dexamethasone suppresses the expression of HSL mRNA in a concentration dependent manner in mammary epithelial cells. GH has slightly effect and did not have a concentration-dependency effect on the expression of HSL mRNA in a physiological concentration. During late pregnancy, insulin sensitivity is increased in rat mammary gland [15], accompanied by elevation plasma insulin levels [16]. Plasma fatty acids concentration is also elevated during late pregnancy [17]. Probably, during mammogensis and lactation, mammary epithelia cells actively adapt to nutritional and hormonal conditions.

In conclusion, we demonstrated that saturated LCFAs (palmitate and stearate) stimulates, and that insulin, dexamethasone and GH suppress the expression of HSL mRNA in bovine mammary epithelial cells, suggesting that HSL is regulated by nutrition and hormones and may be play an important role in lipid and energy metabolism in lactating mammary gland.

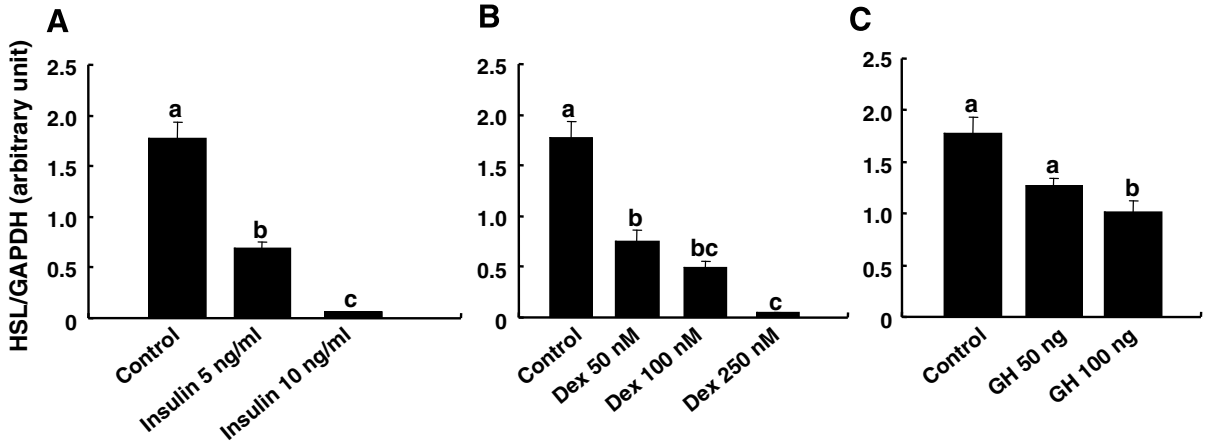

Fig. 3. Effects of insulin, dexamethasone and bovine growth hormone on the expression of bovine HSL mRNA in bMEC. The cells were treated with 5 and 10 ng/ml insulin (A), 50–250 μM dexamethasone (Dex) (B) or 50 and 100 ng/ml growth hormone (GH) (C). The values without associated common letters are significantly different ($P < 0.05$, Bonferroni's multiple range test).

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